



DOCKET NO: IBIS0038-103/IBIS-0490  
Title: MASS SPECTROMETRIC METHODS FOR BIOMOLECULAR  
SCREENING  
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Agent: Daniel M. Scolnick Atty Phone: 215 665-2000  
Replacement Sheet 2 of 33

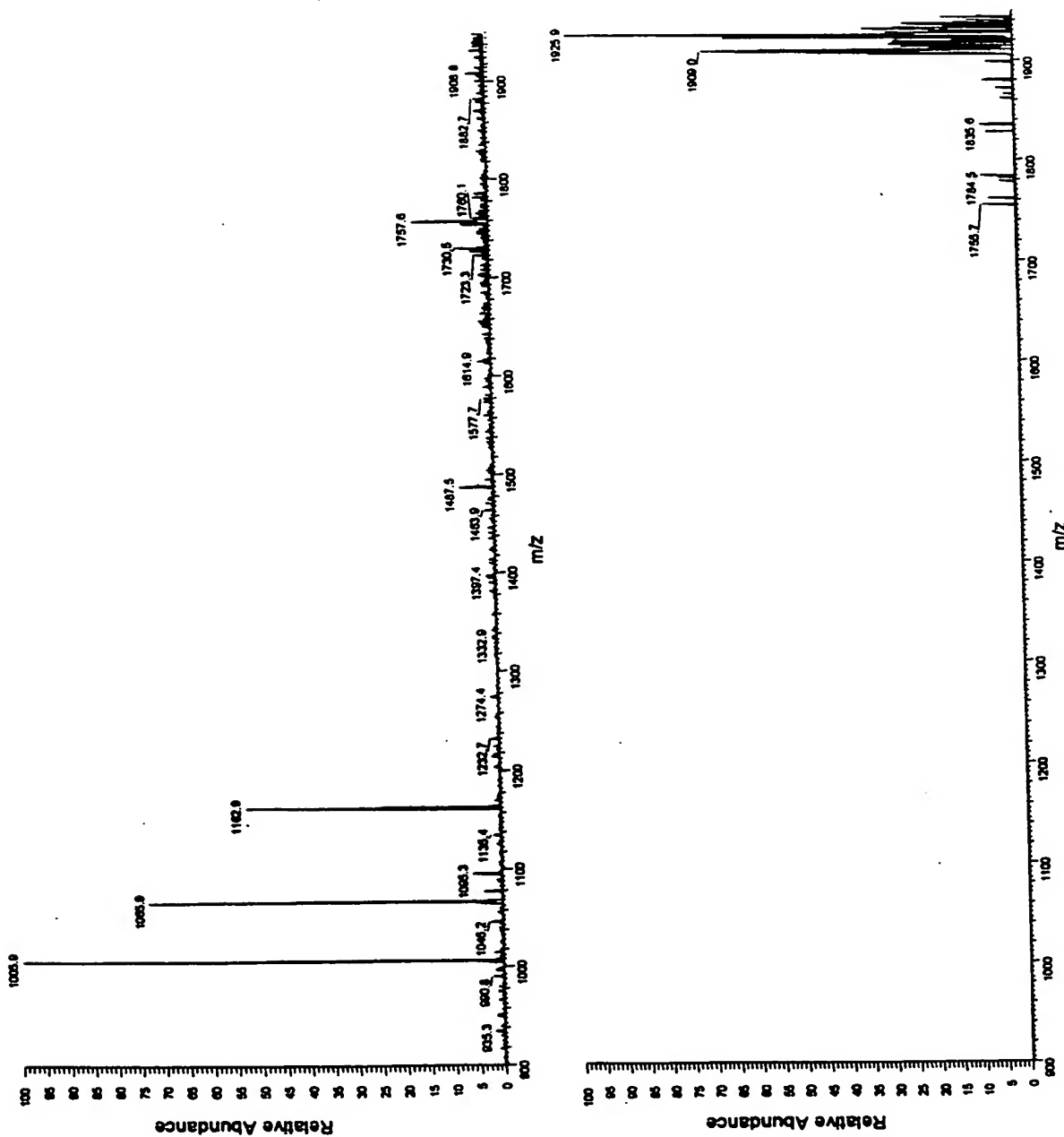


Figure 2. MS/MS of control RNA/DNA (upper); control + paromomycin (lower)

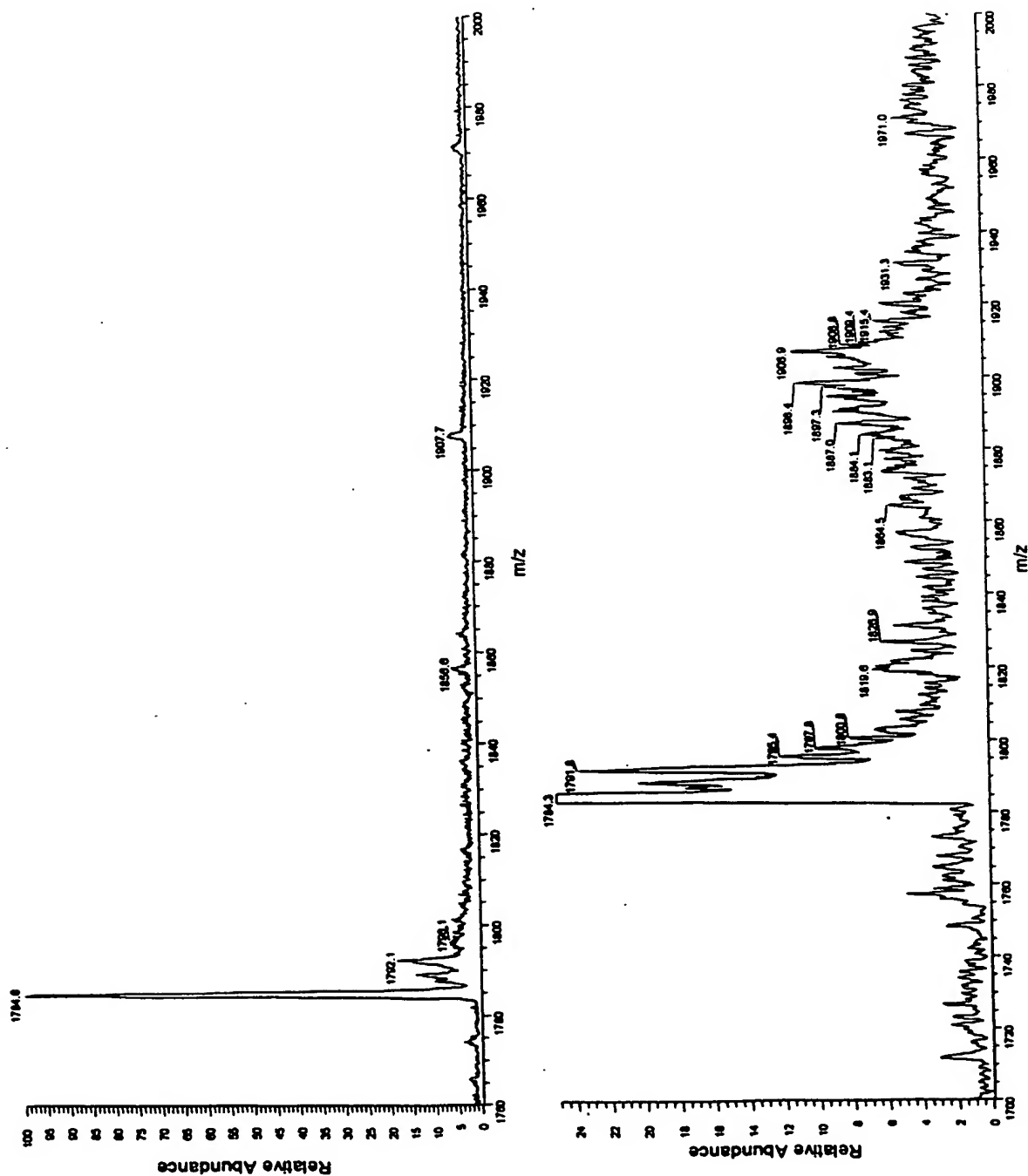


Figure 3. RNA/DNA chimera+paramomycin (upper); chimera+library (lower)

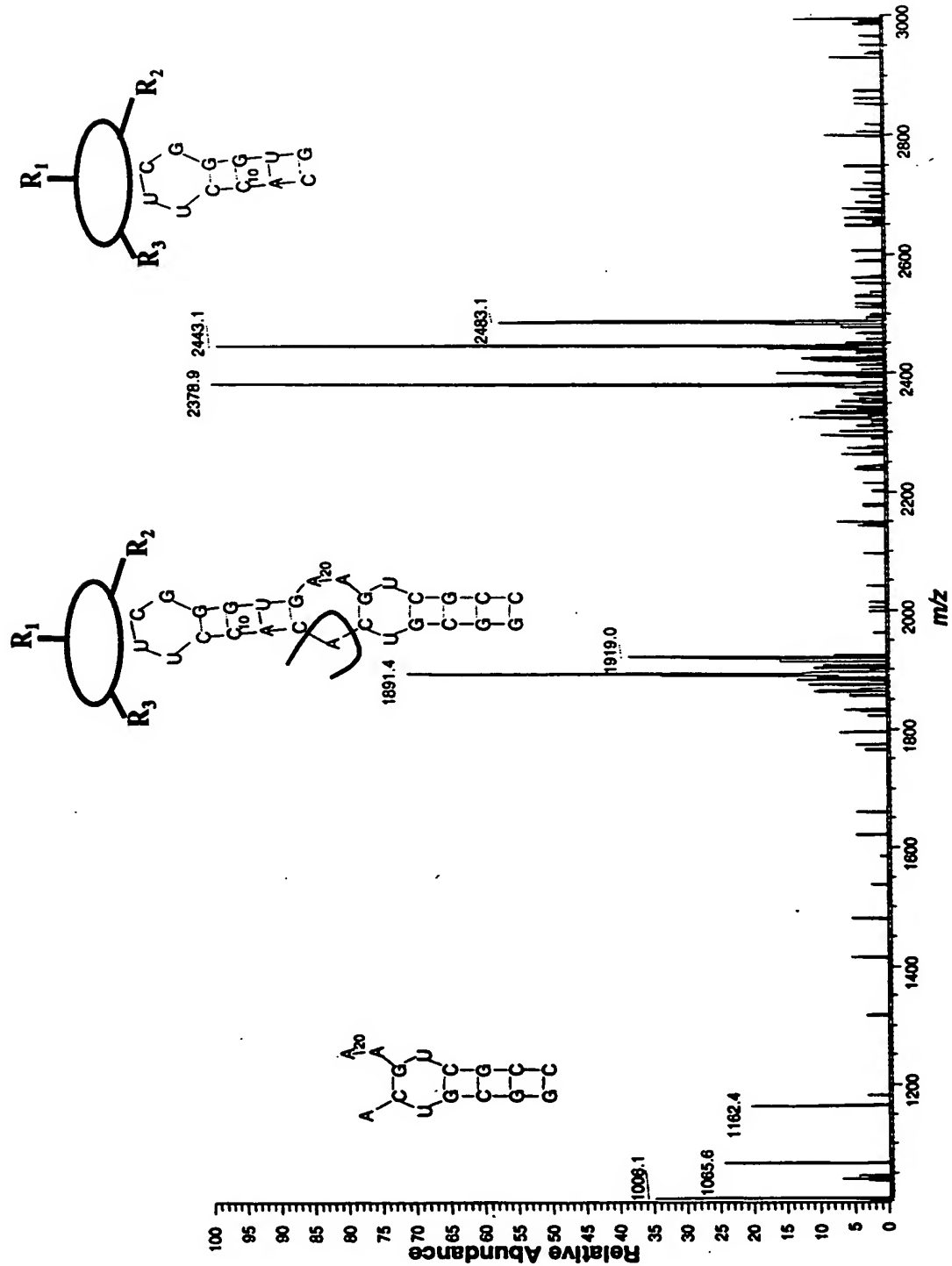


Figure 4. MS-MS analysis of member bound to RNA/DNA chimera

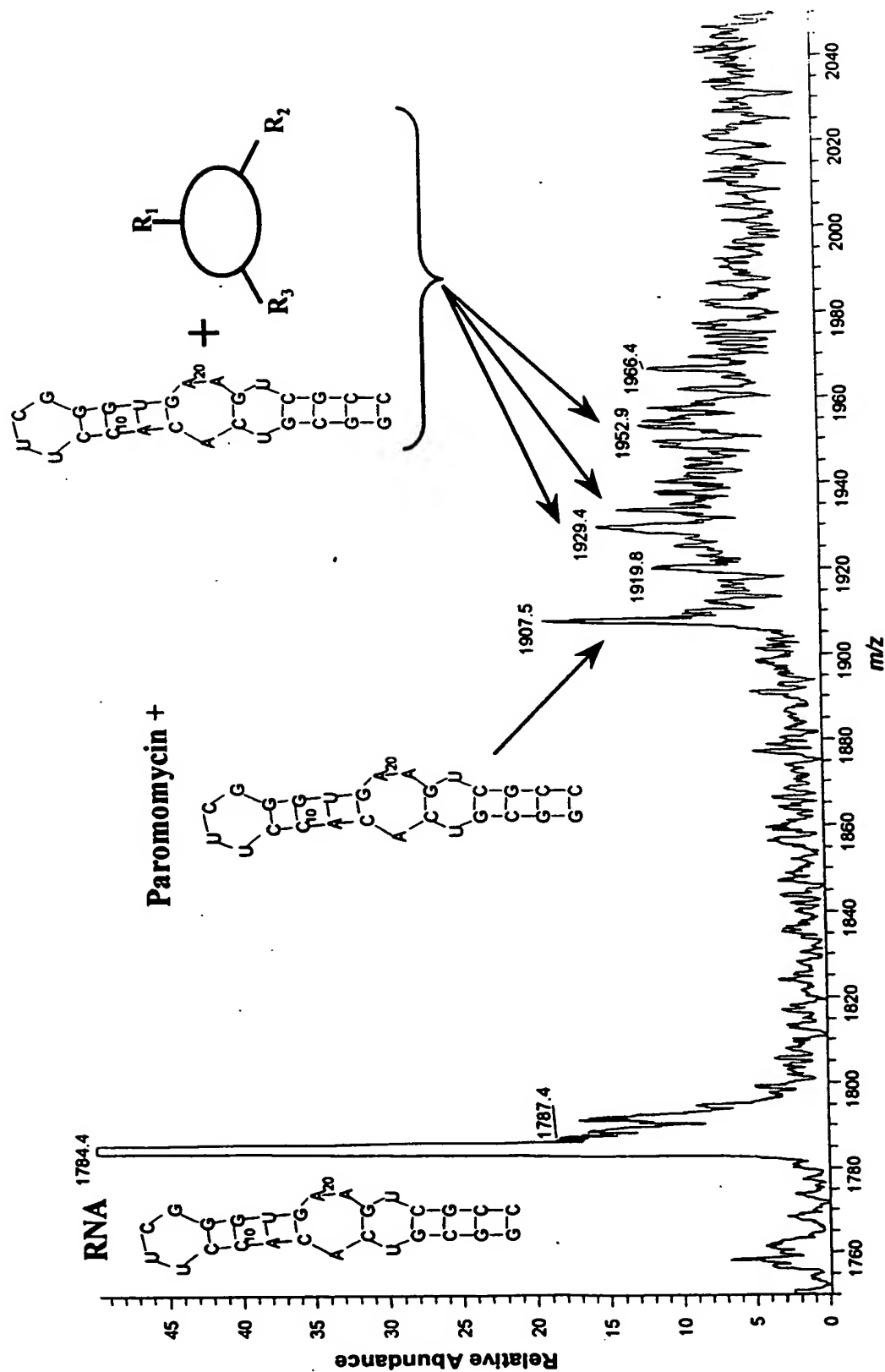


Figure 5. ESI-MS of RNA/DNA chimera bound to paromomycin and library

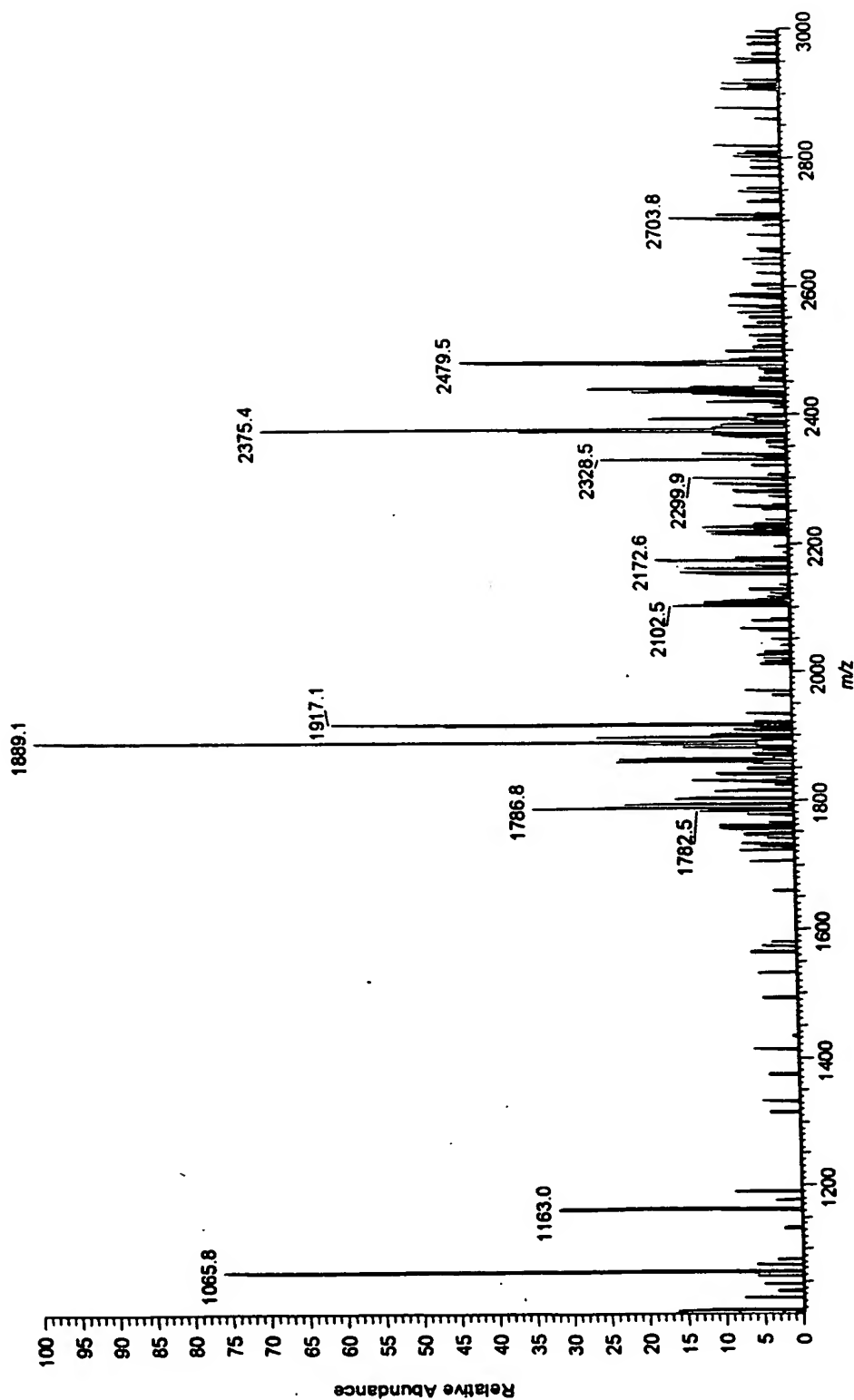


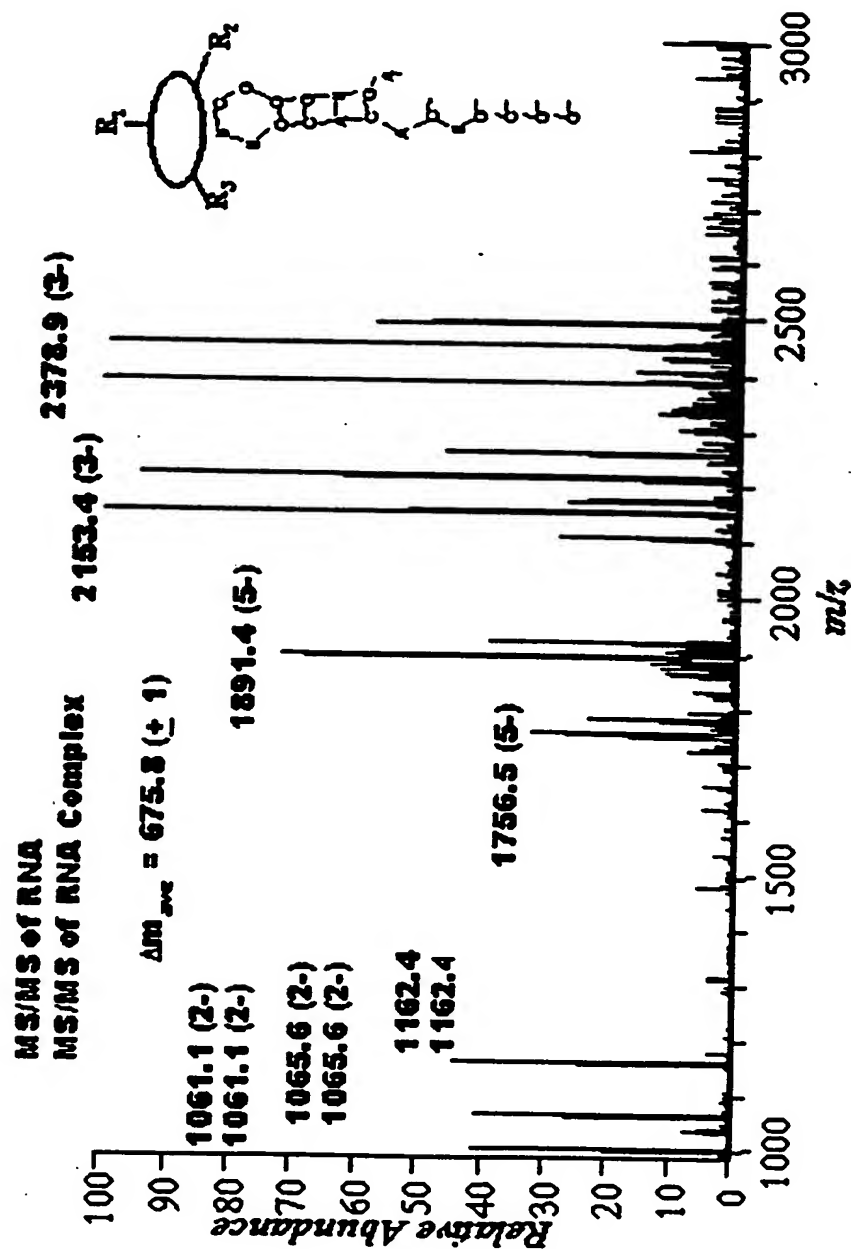
Figure 6. MS/MS of RNA/DNA chimera + compound with mass 665.1 not bound at the A-site

**Figure 7. MS-MS analysis of member bound to RNA/DNA chimera at the A-Site**

# MASS Analysis of Binding Location

non-A site binder

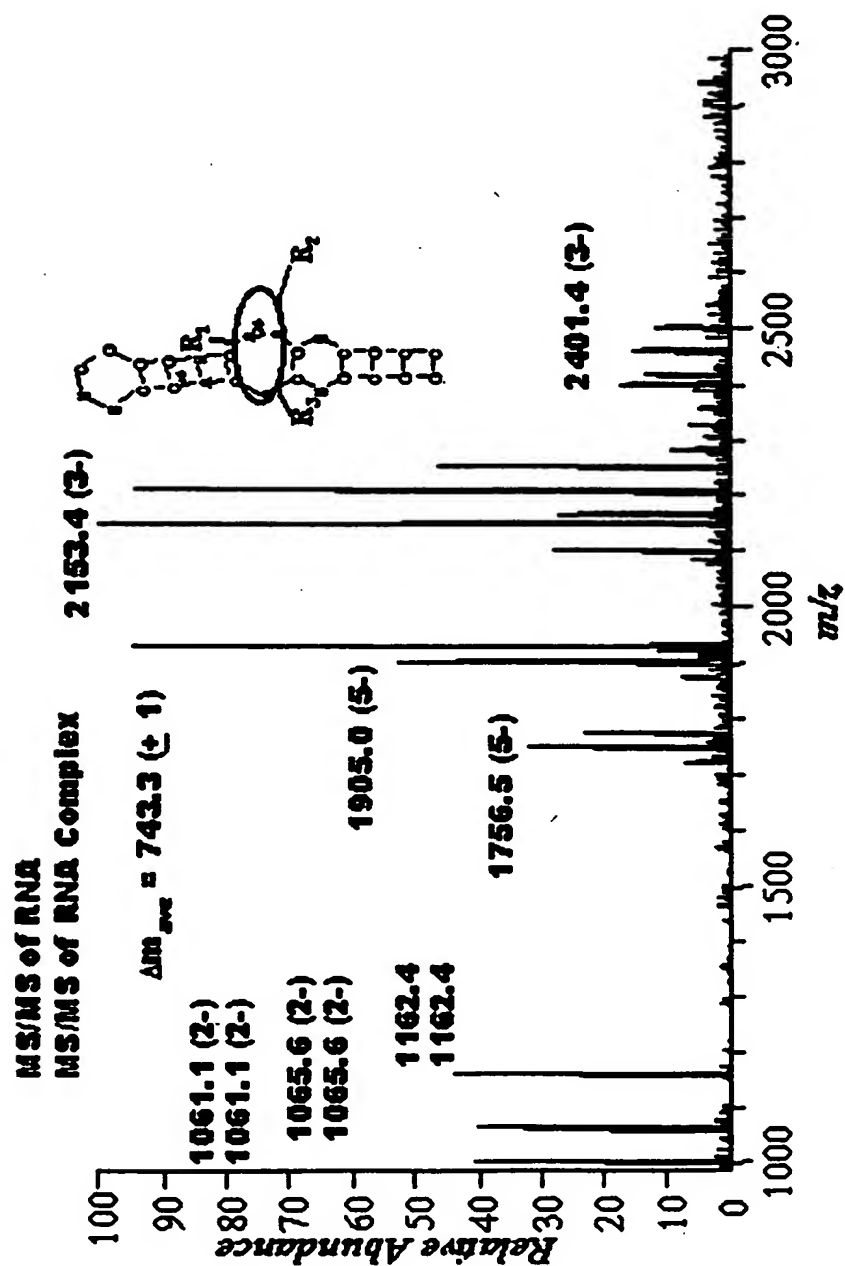
FIGURE 10



# MASS Analysis of Binding Location

non-A site binder

FIGURE 11





**MASS analysis of 16S A site RNA plus  
 216 member library  
 (performed on quadrupole ion trap)**

**FIGURE 12**

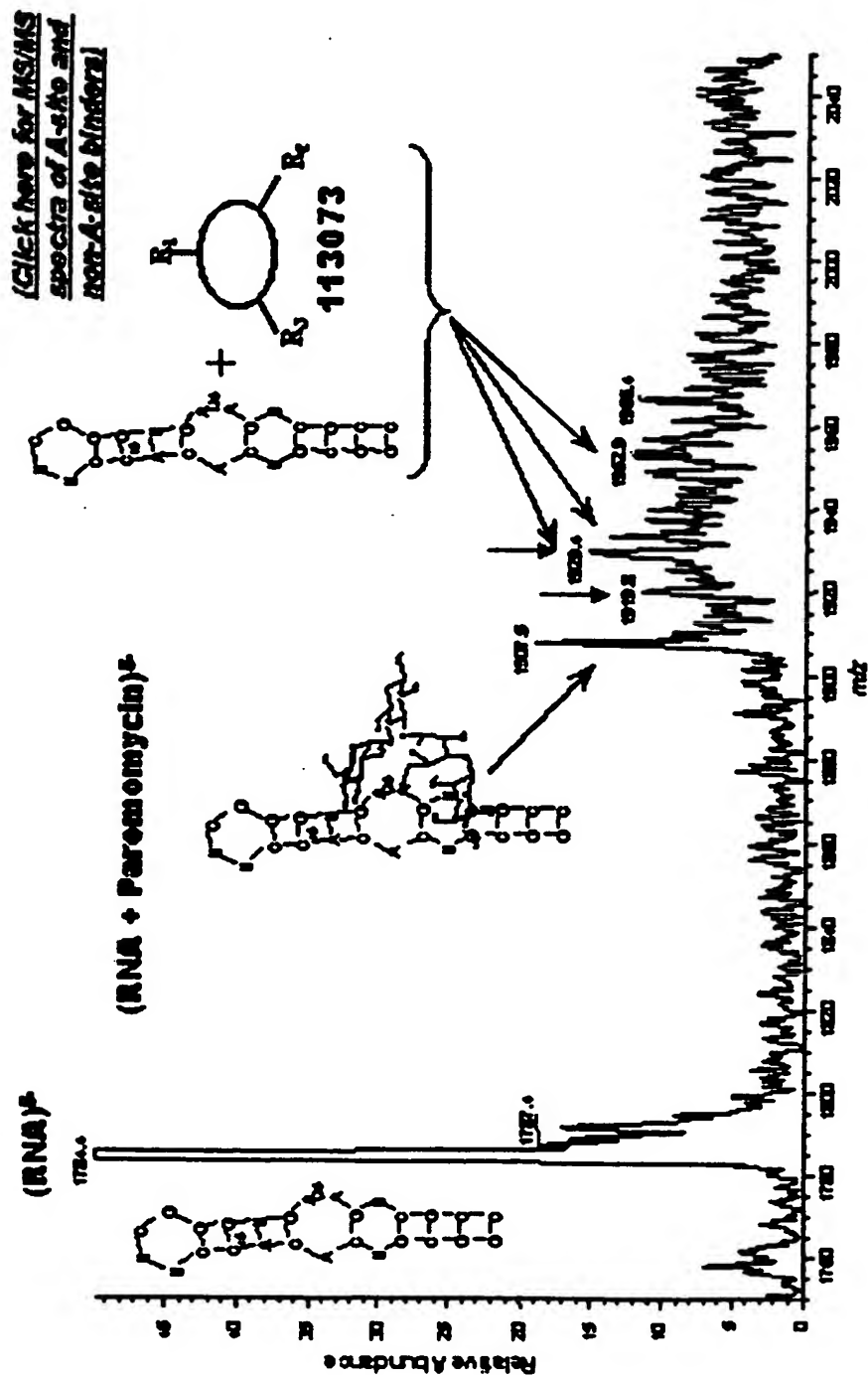


Figure 13

# High Precision ESI-FTICR Mass Measurement of 16S A site RNA/Paromomycin Complex

use of unbound RNA as internal mass standard  
 provides low ppm mass measurement errors

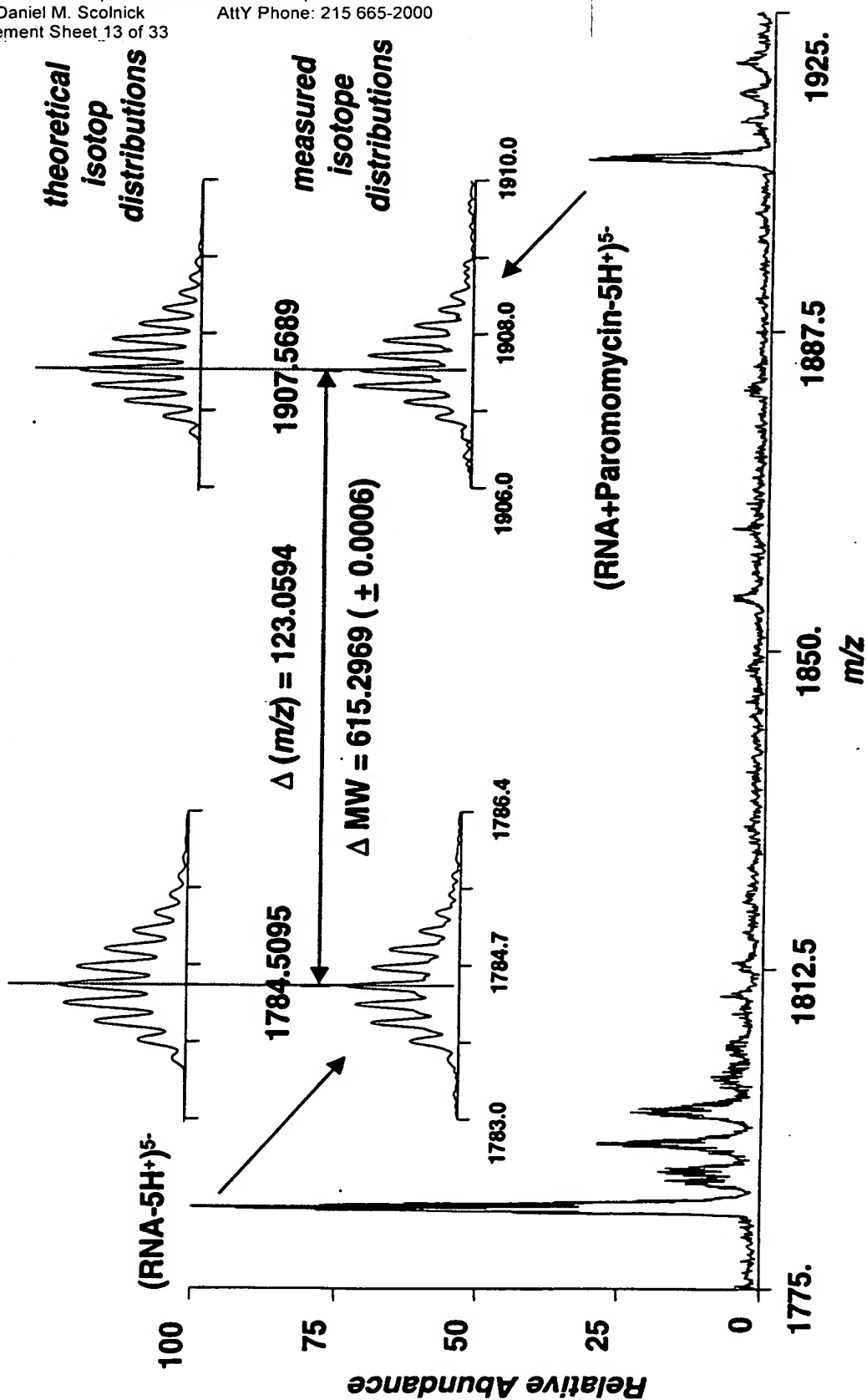
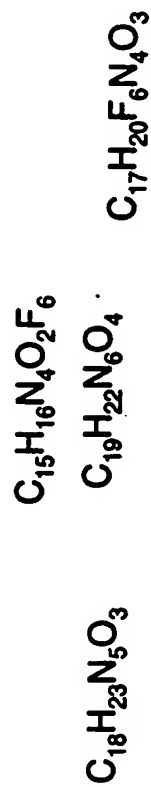


Figure 14

# MASS of 60-Member Ibis Library Against 16S A-site RNA



Free 16S  
RNA

Complexes

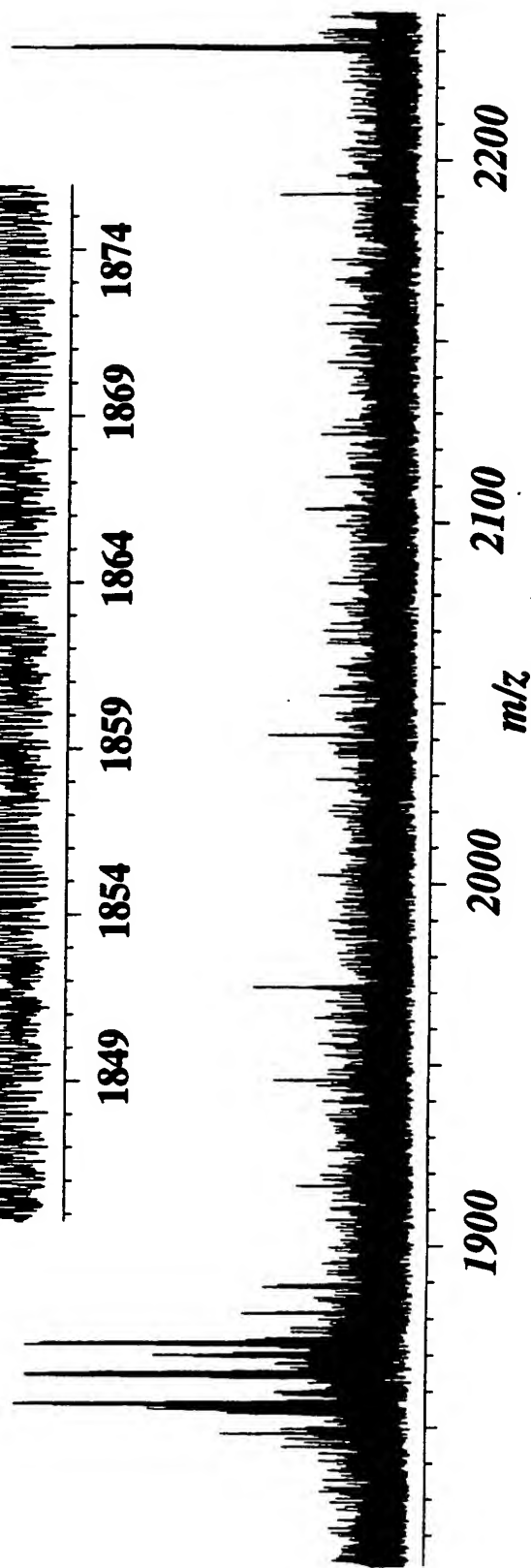


Figure 15

# MASS of 60-member Library against 16S A-site Model

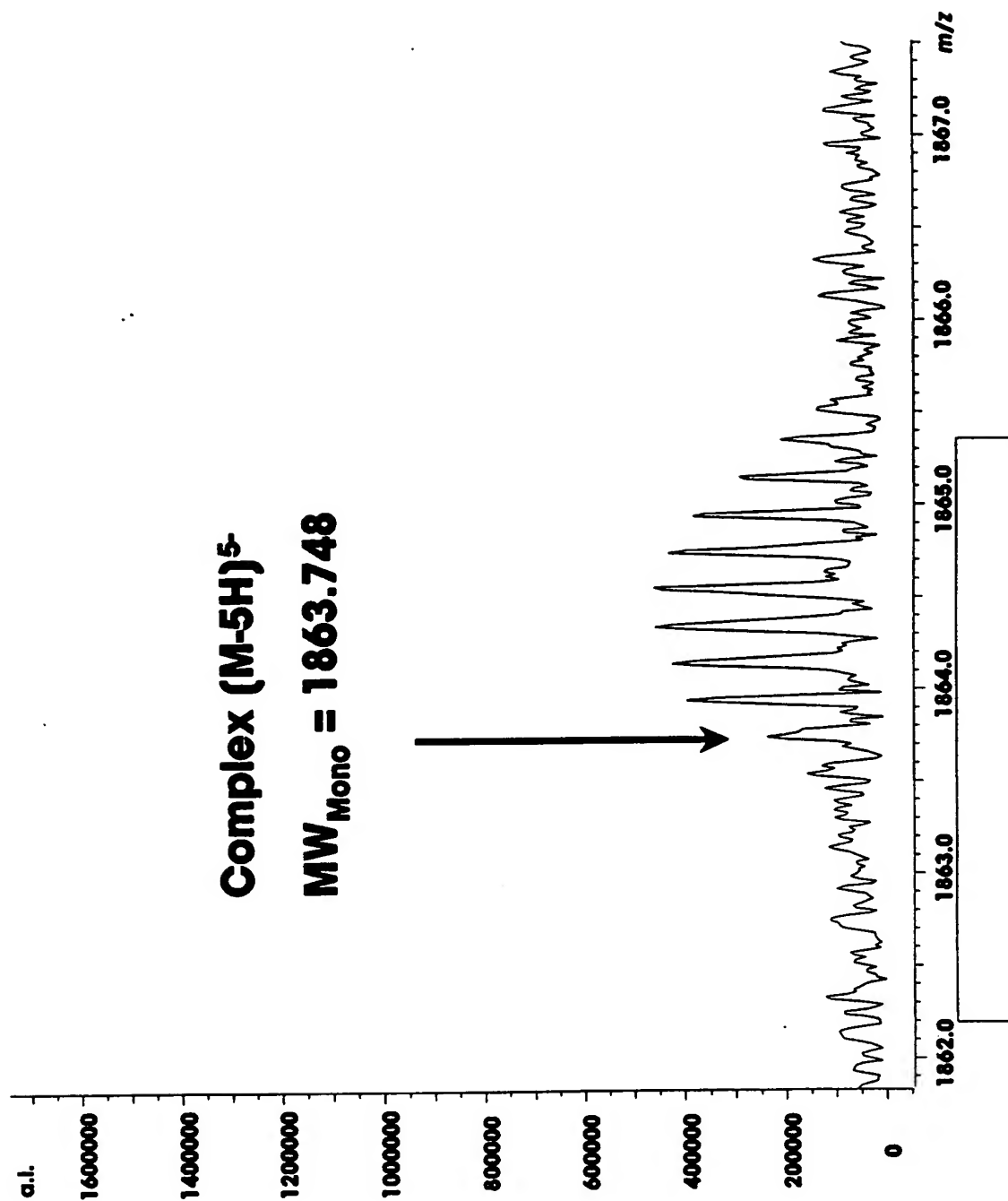
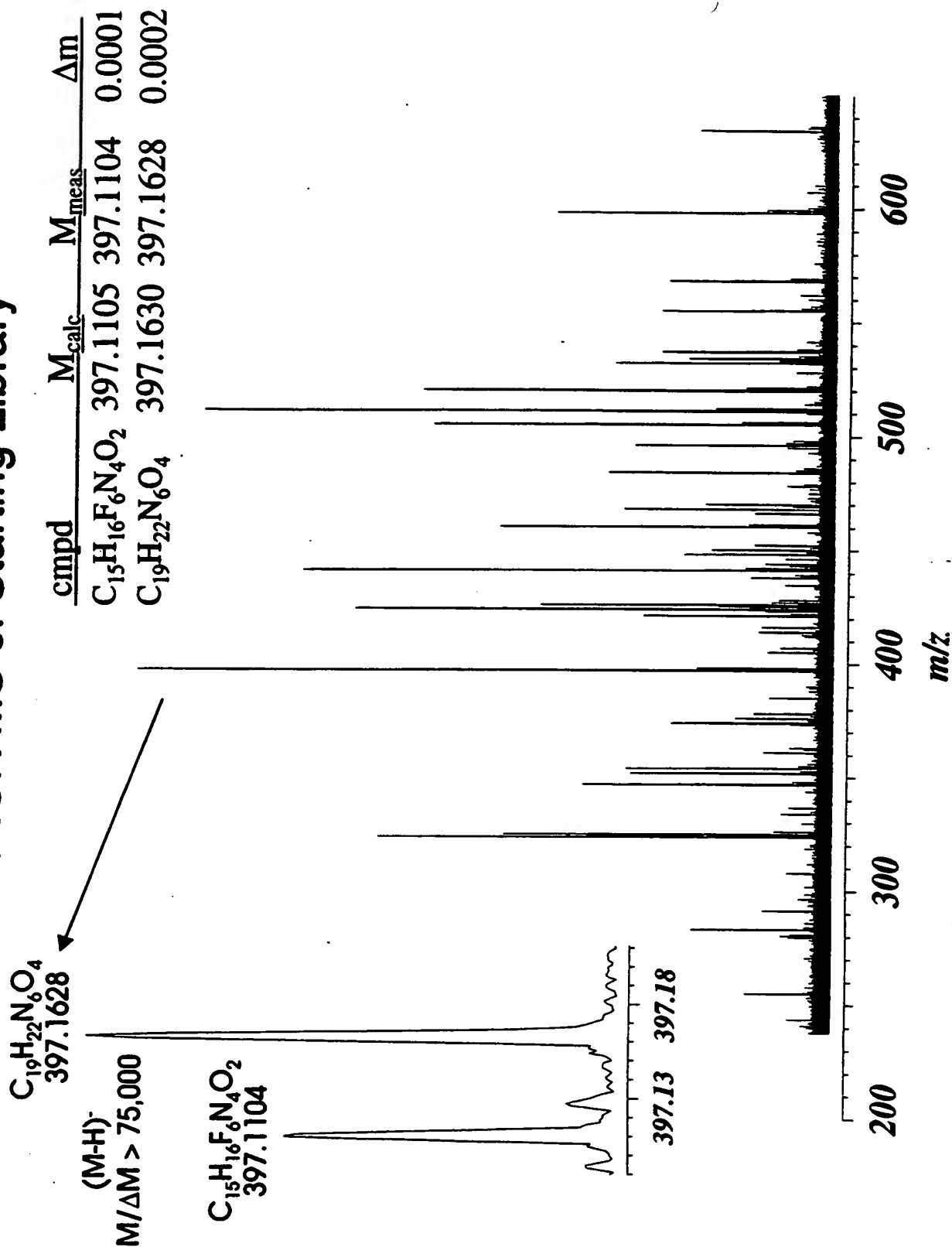


Figure 16

# FT-ICR MS of Starting Library



**Figure 17**  
**Compound Identification from a 60-member**  
**Combinatorial library with MASS**

<b>Complex <math>M_{meas}</math></b>	<b>9320.300<math>\pm</math>.009 Da</b>
<b>RNA <math>M_{meas}</math></b>	<b>8922.189<math>\pm</math>.009</b>
<b><math>\Delta M</math></b>	<b>398.111<math>\pm</math>.009 Da</b>

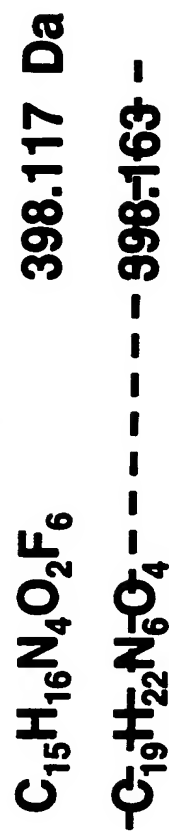


Figure 18

# Elemental Composition Constraints

Measured Mass: 615.2969

Mass Tolerance: 1.0 ppm

Charge: 0

Element Min. atoms Max. atoms

<sup>12</sup> C	12	30
<sup>1</sup> H	23	60
<sup>16</sup> O	7	20
<sup>14</sup> N	3	20

Possible Elemental Compositions:

Calc. Mass (amu)	Error (ppm)	Molecular Formula
---------------------	----------------	-------------------

615.296291	0.98	<sup>16</sup> O <sub>4</sub> <sup>14</sup> N <sub>19</sub> <sup>12</sup> C <sub>21</sub> <sup>1</sup> H <sub>33</sub>
615.296298	0.98	<sup>16</sup> O <sub>9</sub> <sup>14</sup> N <sub>12</sub> <sup>12</sup> C <sub>22</sub> <sup>1</sup> H <sub>39</sub>
615.296305	0.97	<sup>16</sup> O <sub>14</sub> <sup>14</sup> N <sub>5</sub> <sup>12</sup> C <sub>23</sub> <sup>1</sup> H <sub>45</sub>
615.296808	0.15	<sup>16</sup> O <sub>15</sub> <sup>14</sup> N <sub>17</sub> <sup>12</sup> C <sub>8</sub> <sup>1</sup> H <sub>41</sub>
615.296815	0.14	<sup>16</sup> O <sub>20</sub> <sup>14</sup> N <sub>10</sub> <sup>12</sup> C <sub>9</sub> <sup>1</sup> H <sub>47</sub>

Further constrain by  
 elemental  
 composition of  
 "letters"

unintended  
 products...

"Scaffold"  
 $C_{12}H_{23}N_3O_7$

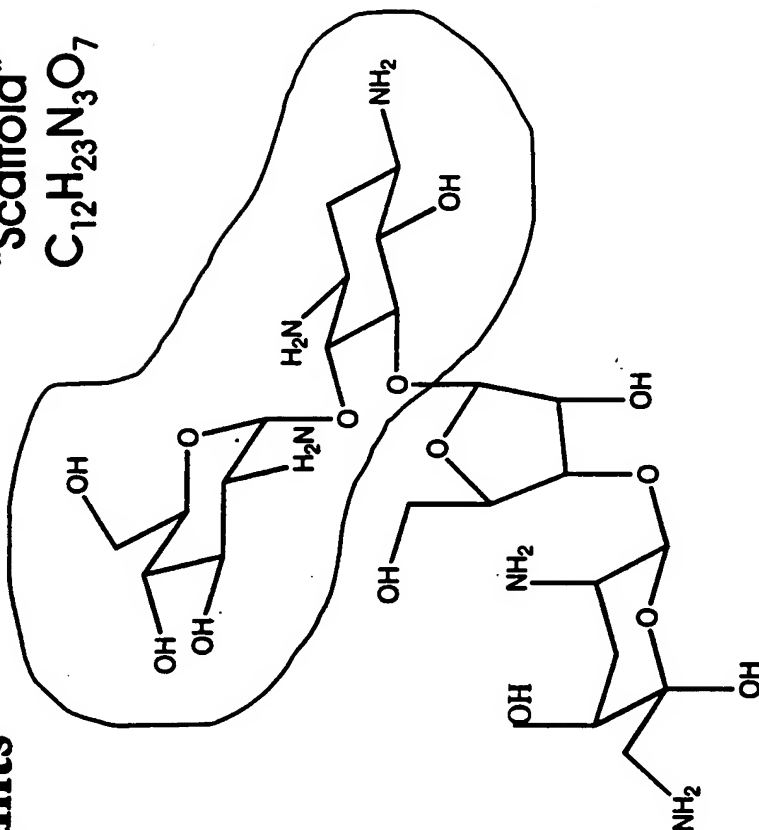
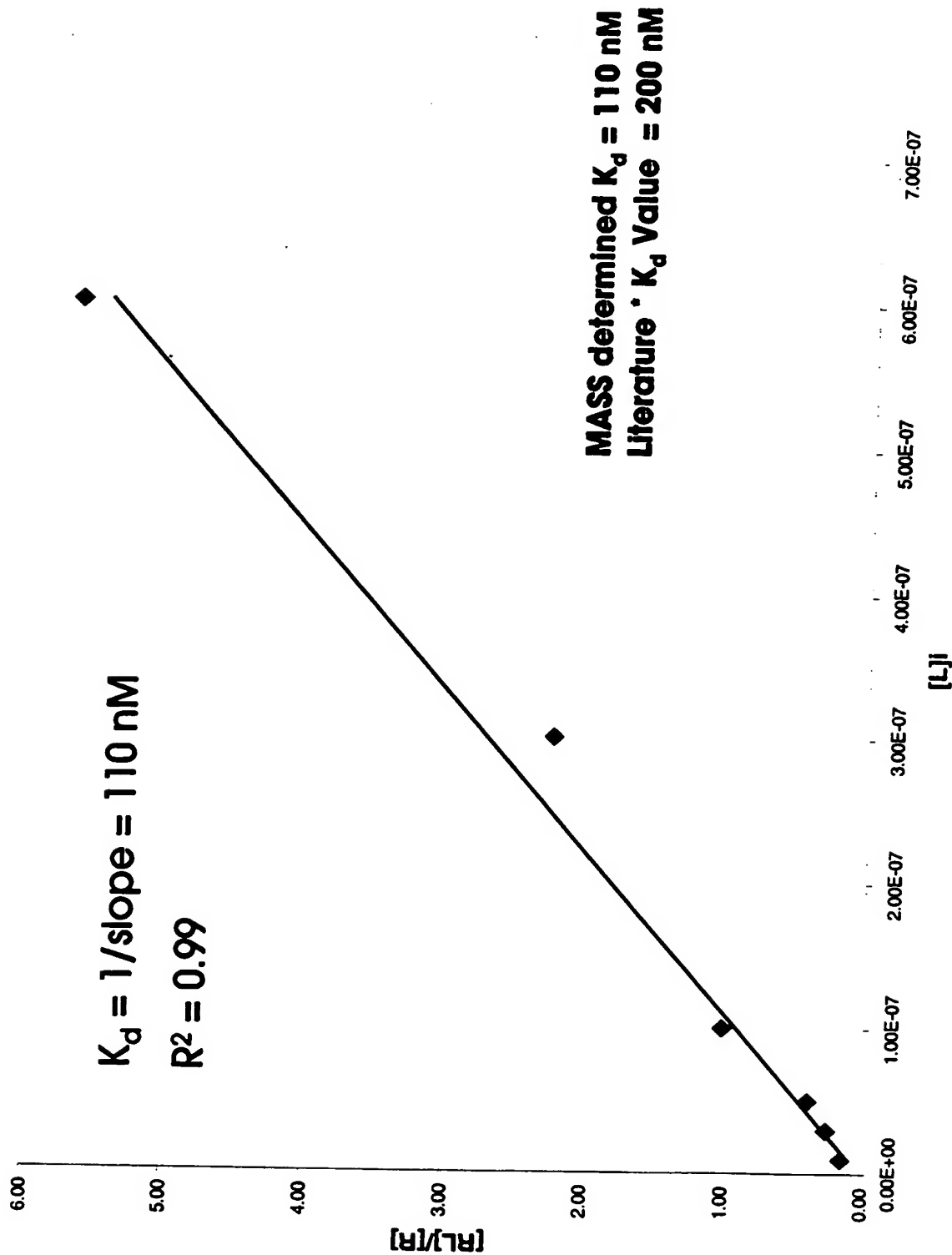


Figure 19

# MASS $K_d$ determination for 16S-Paromomycin





# Figure 20 Multitarget Affinity/Specificity Screening

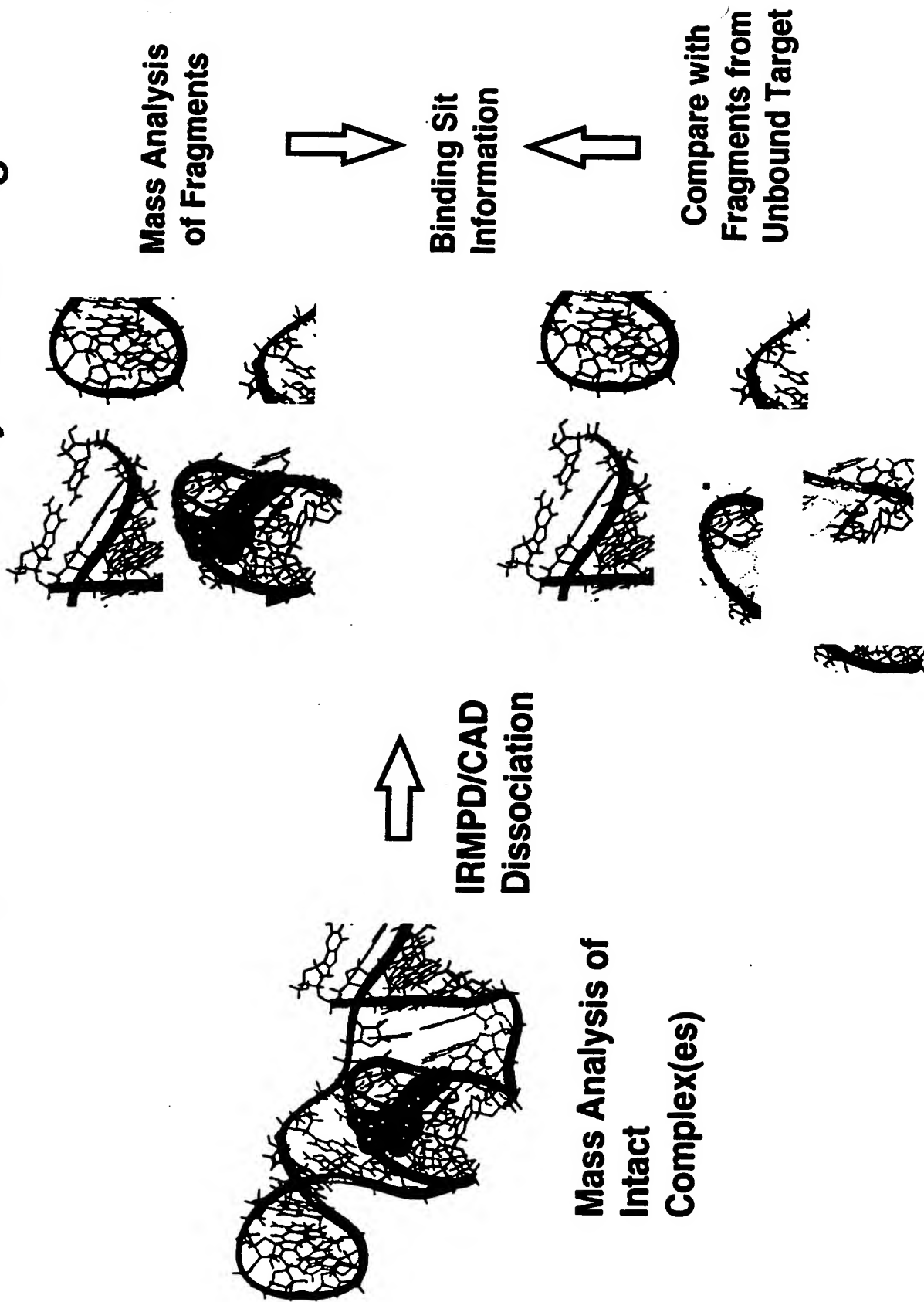


Figure 21

# MASS Analysis of 27 Member Library with 16S A-site RNA

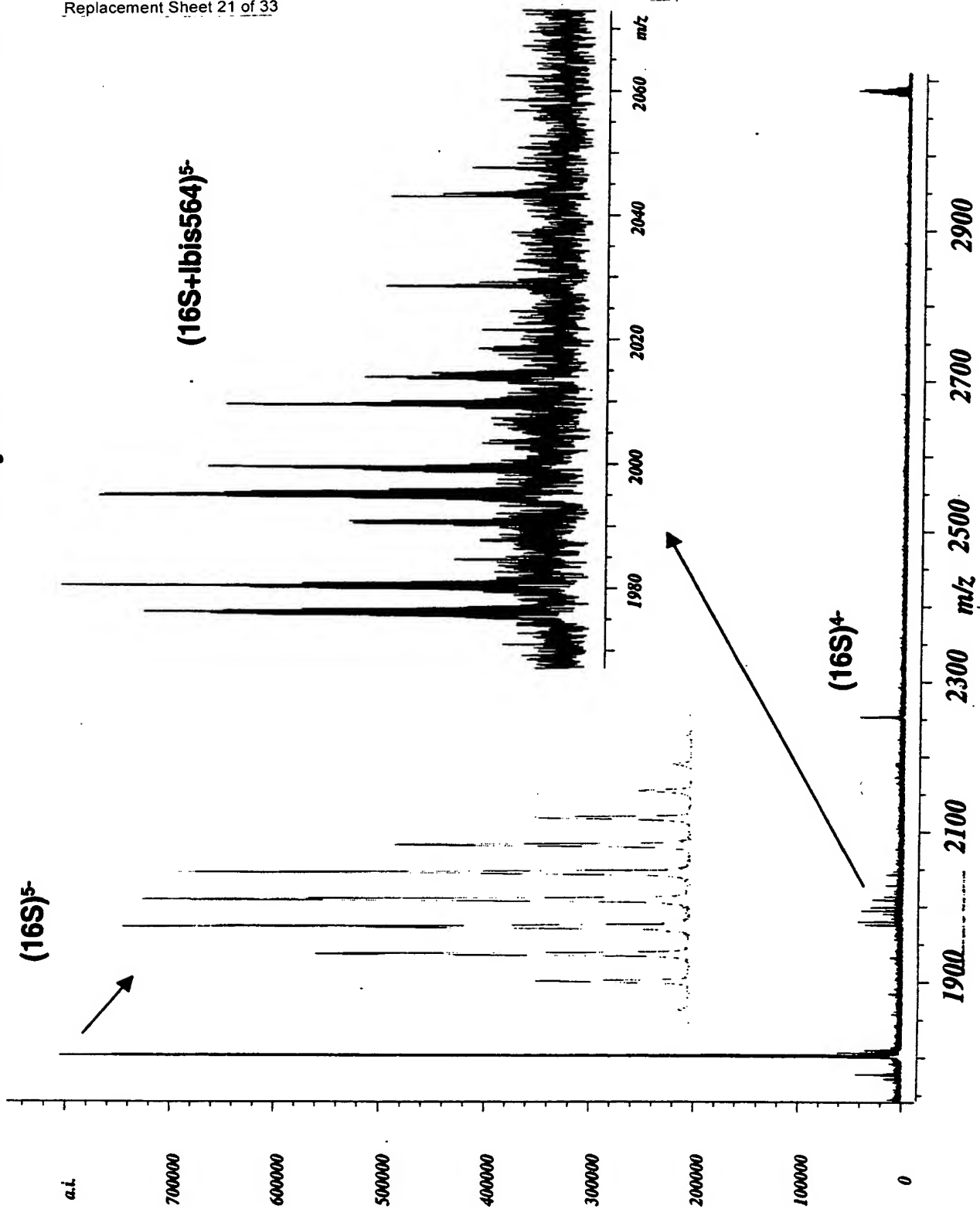
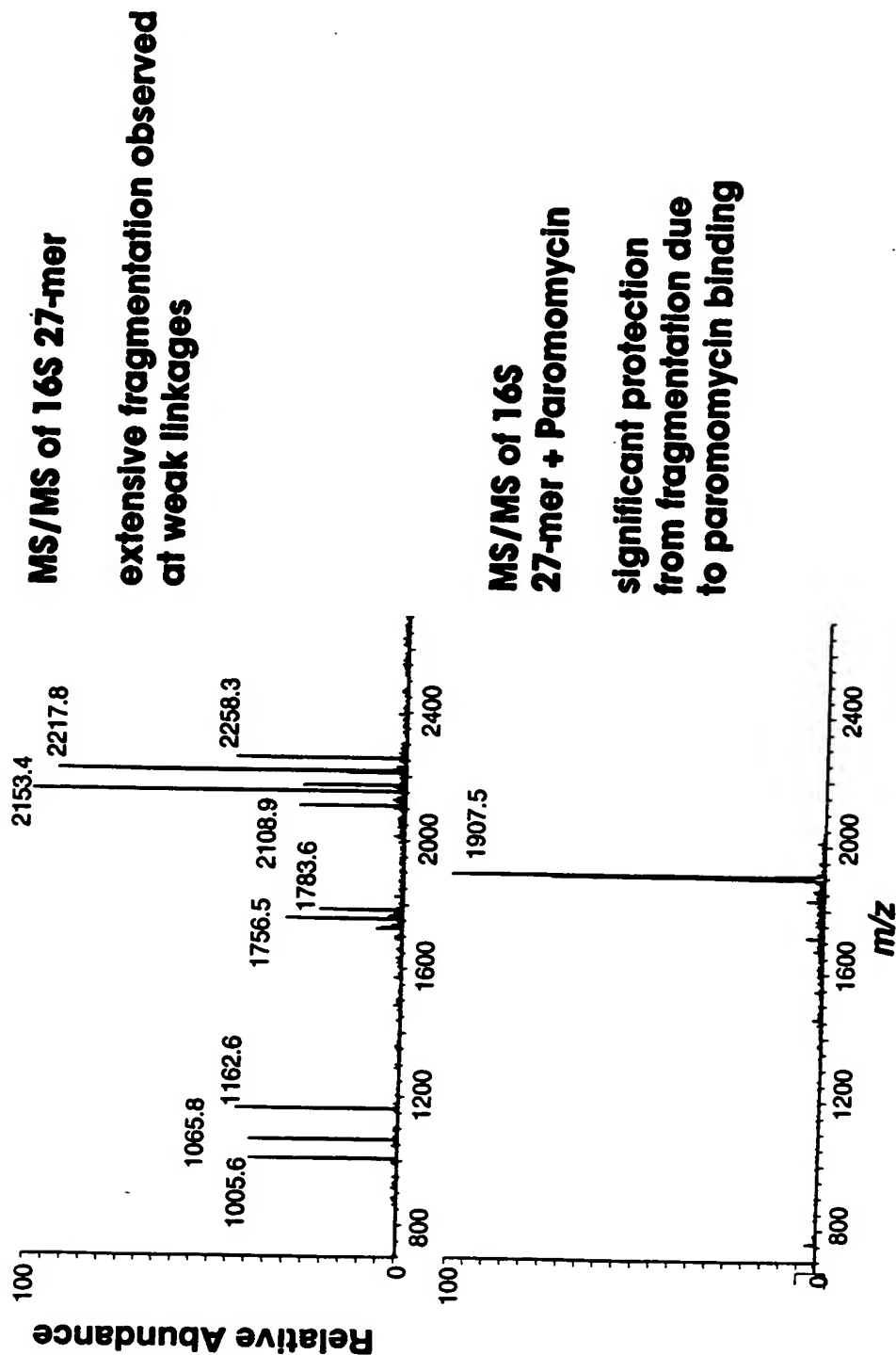


Figure 22

# MASS Protection Assay



# Figure 23 MASS Protection Assay

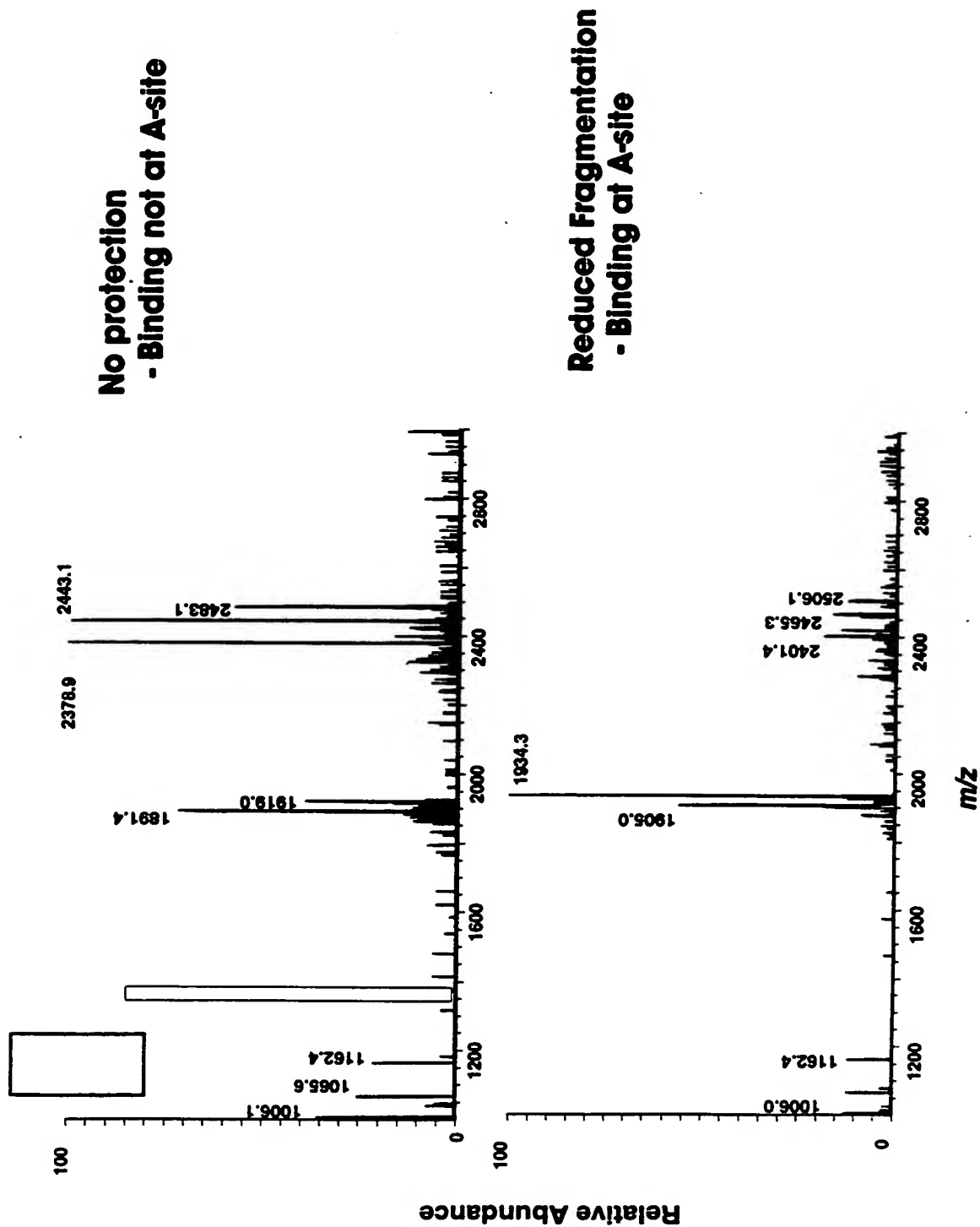
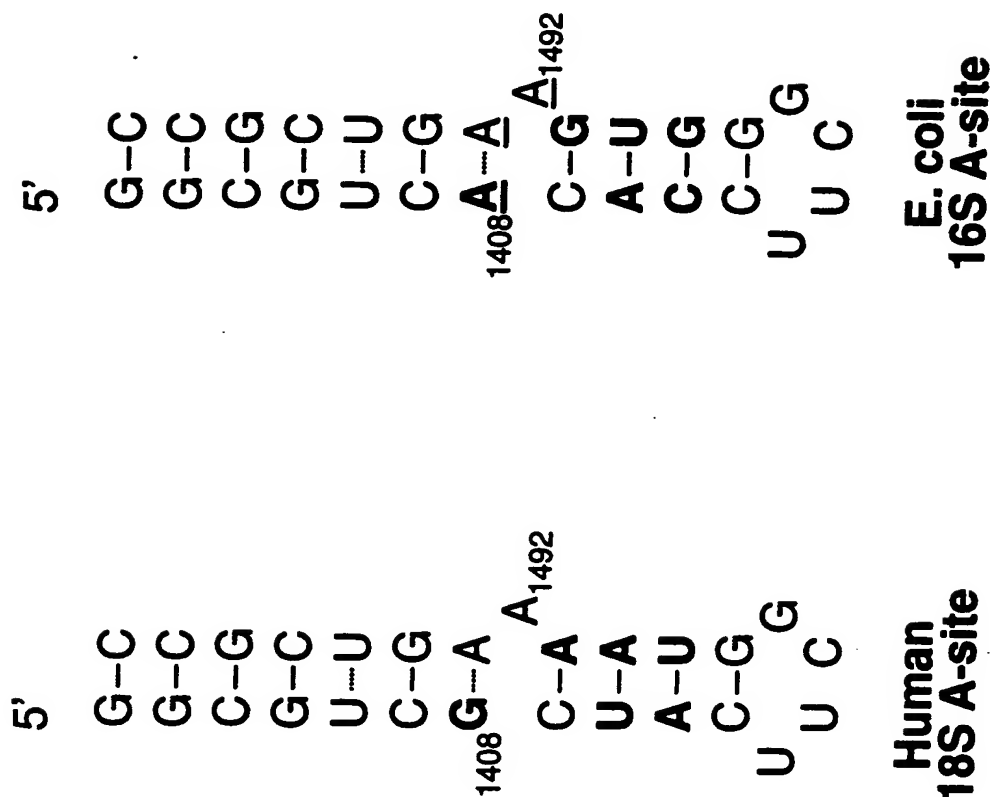


Figure 24

# Eukaryotic and Prokaryotic A-Site

Aminoglycoside antibiotics bind to

A-site of decoding region in 16S RNA



$\Delta$  MW = 15.011 Da

Figure 25  
Neutral Mass Tag Does Not Affect Ligand Binding

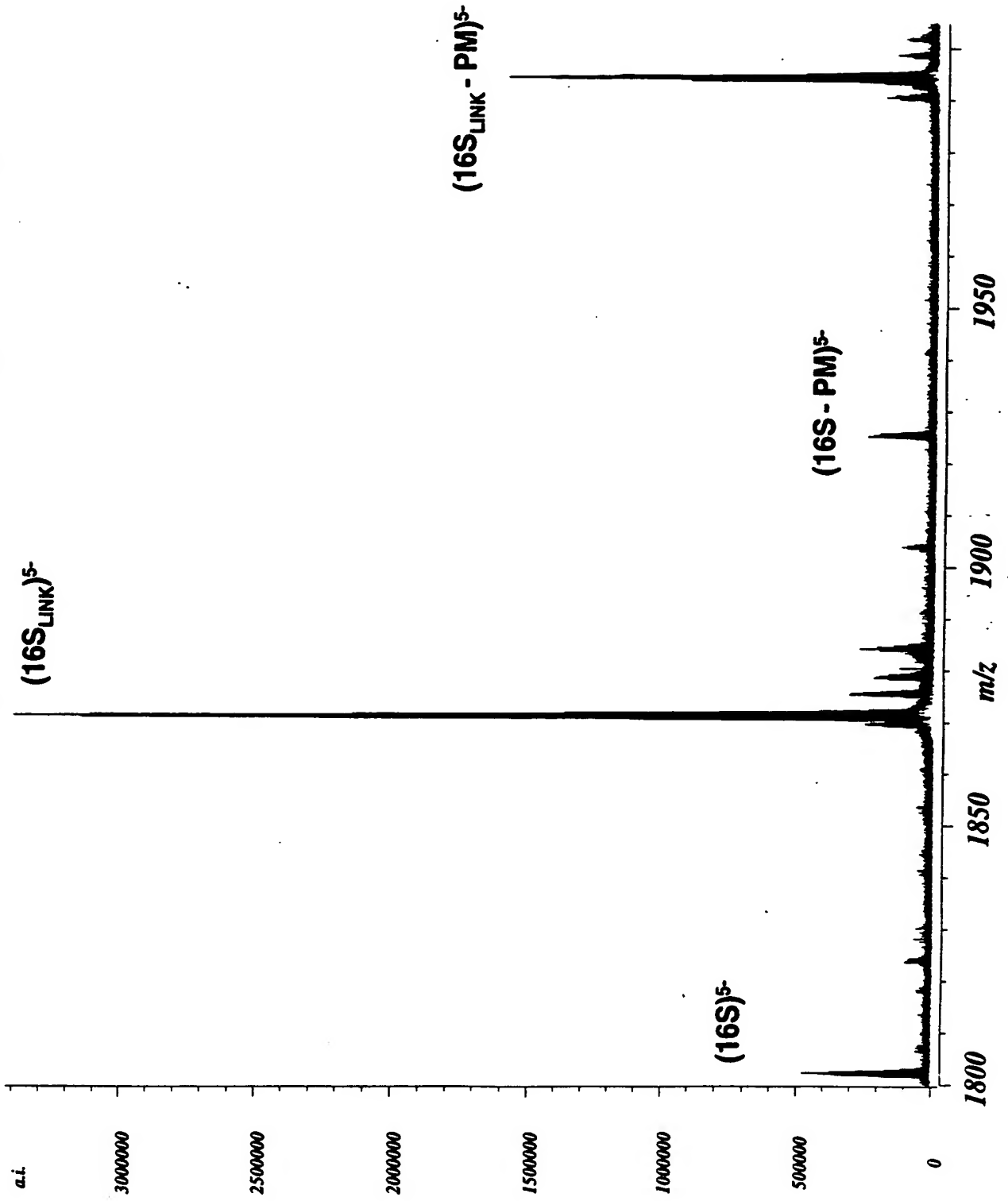
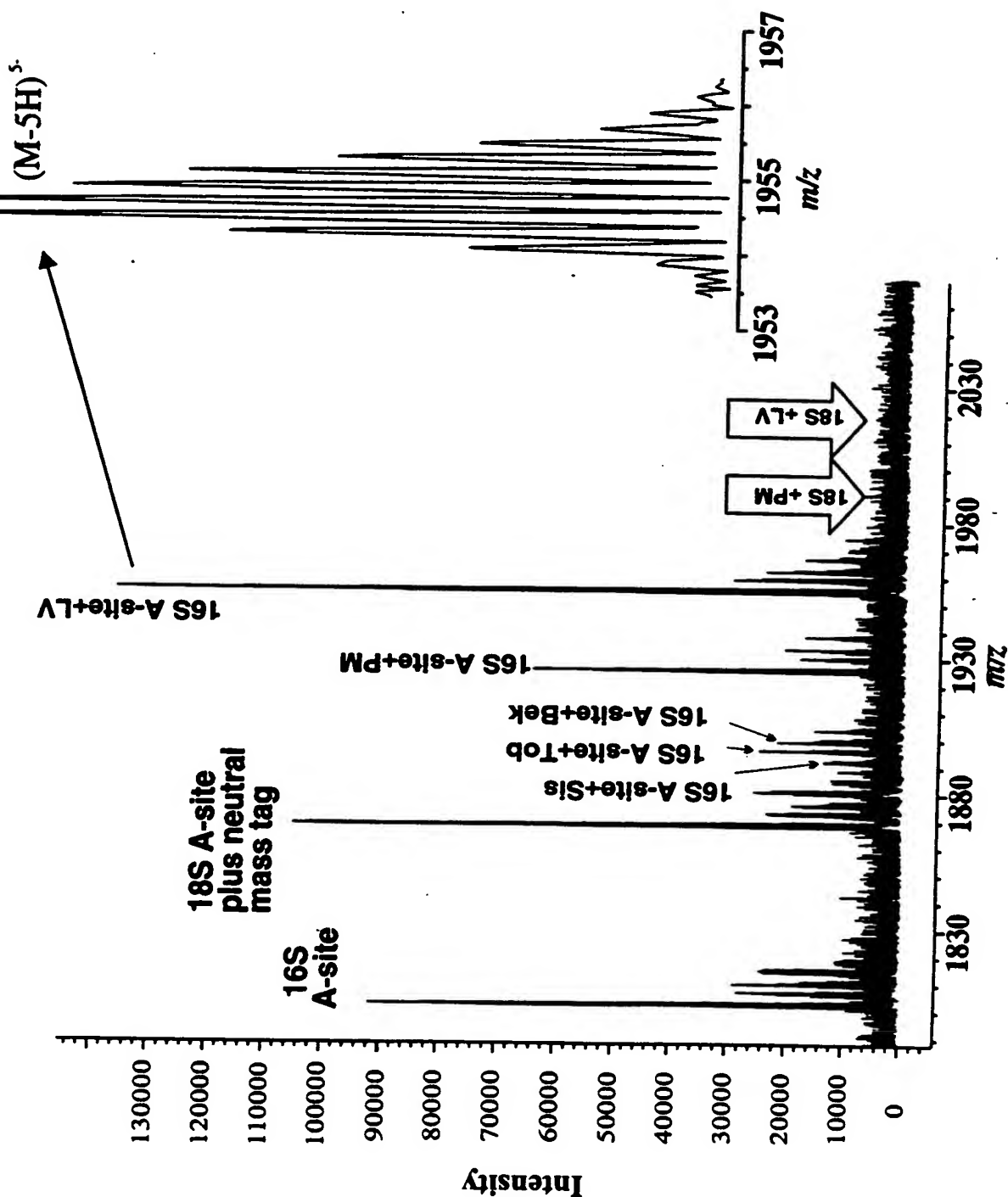


Figure 26

# Simultaneous Screening of 16S A-site and 18S A-site Model RNAs Against Aminoglycoside Mixture



**Figure 27**

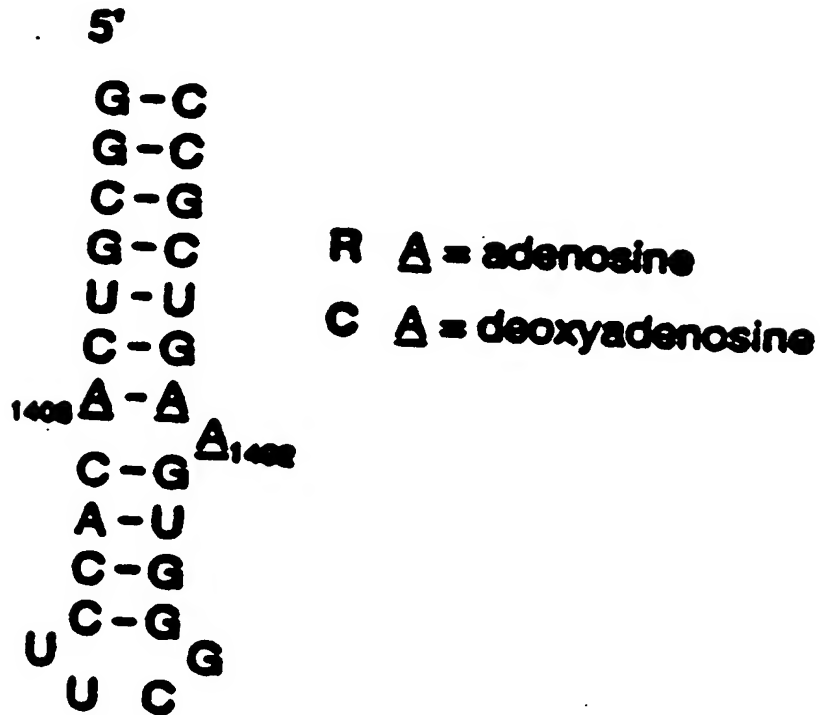




Figure 28

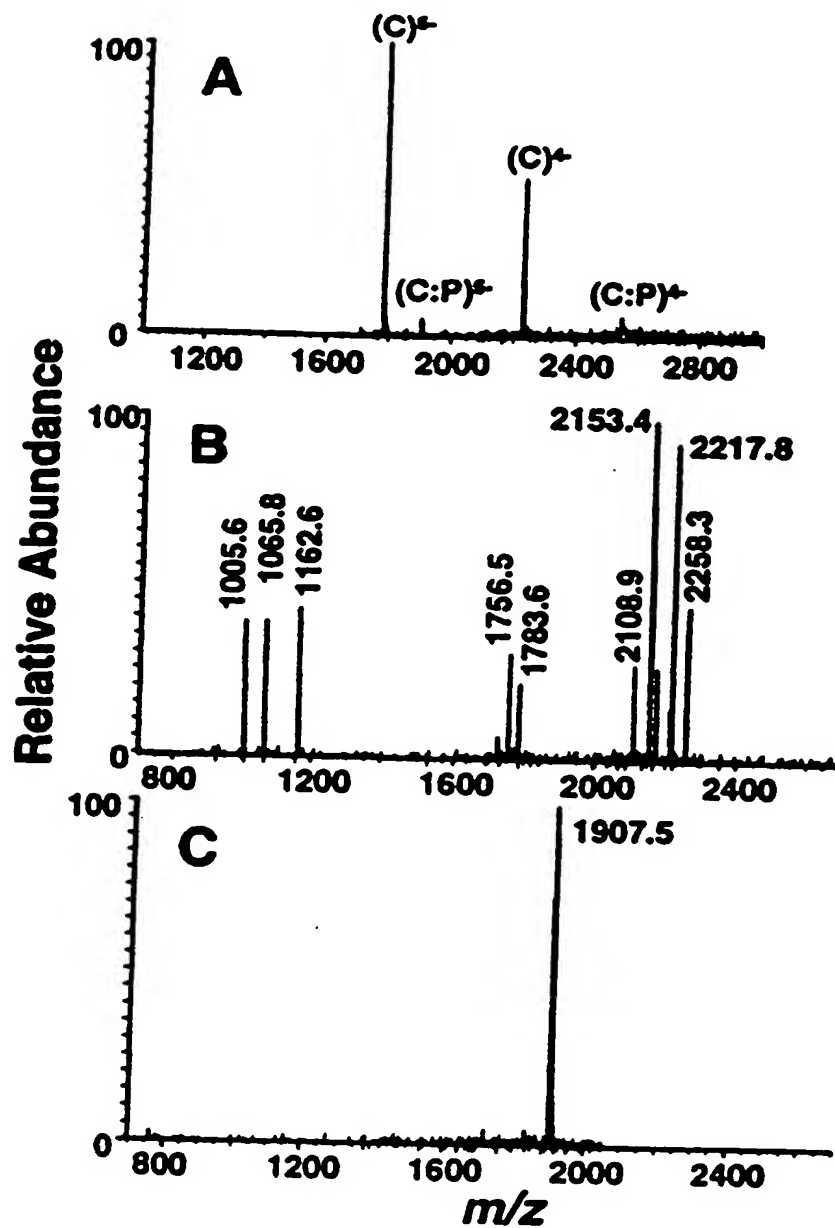
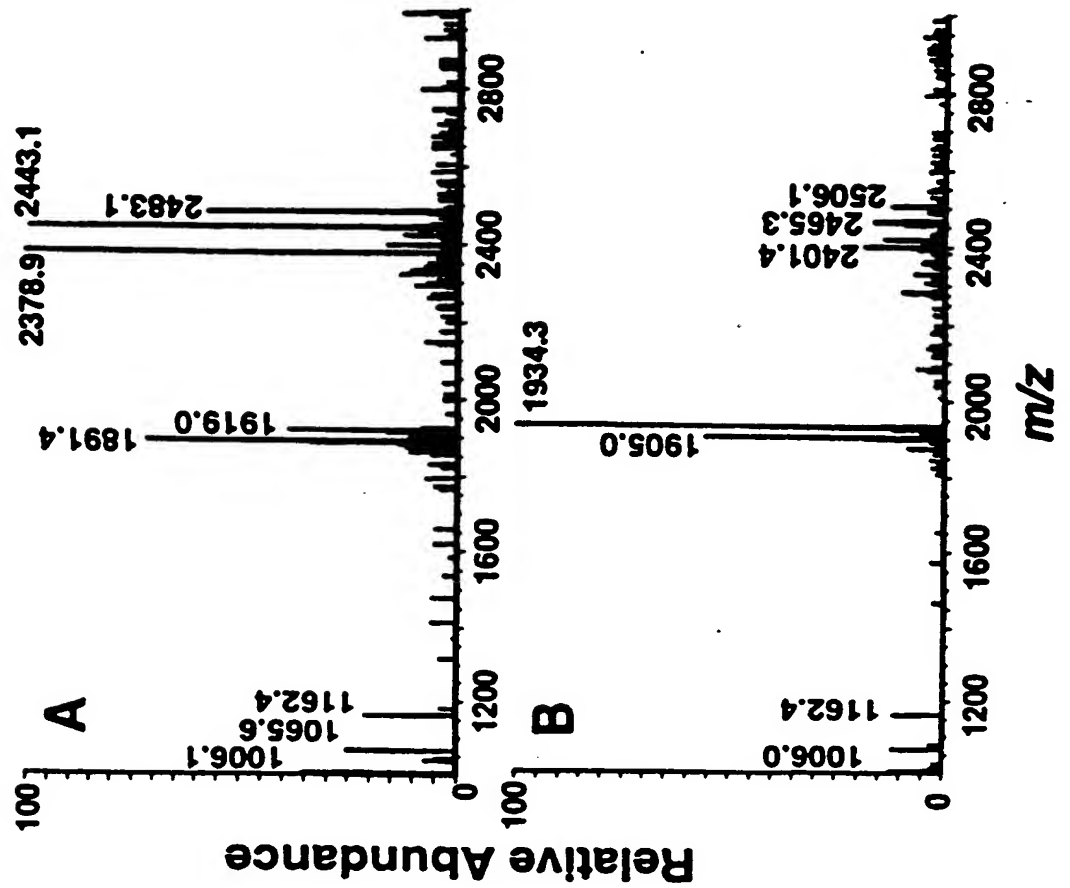


Figure 29



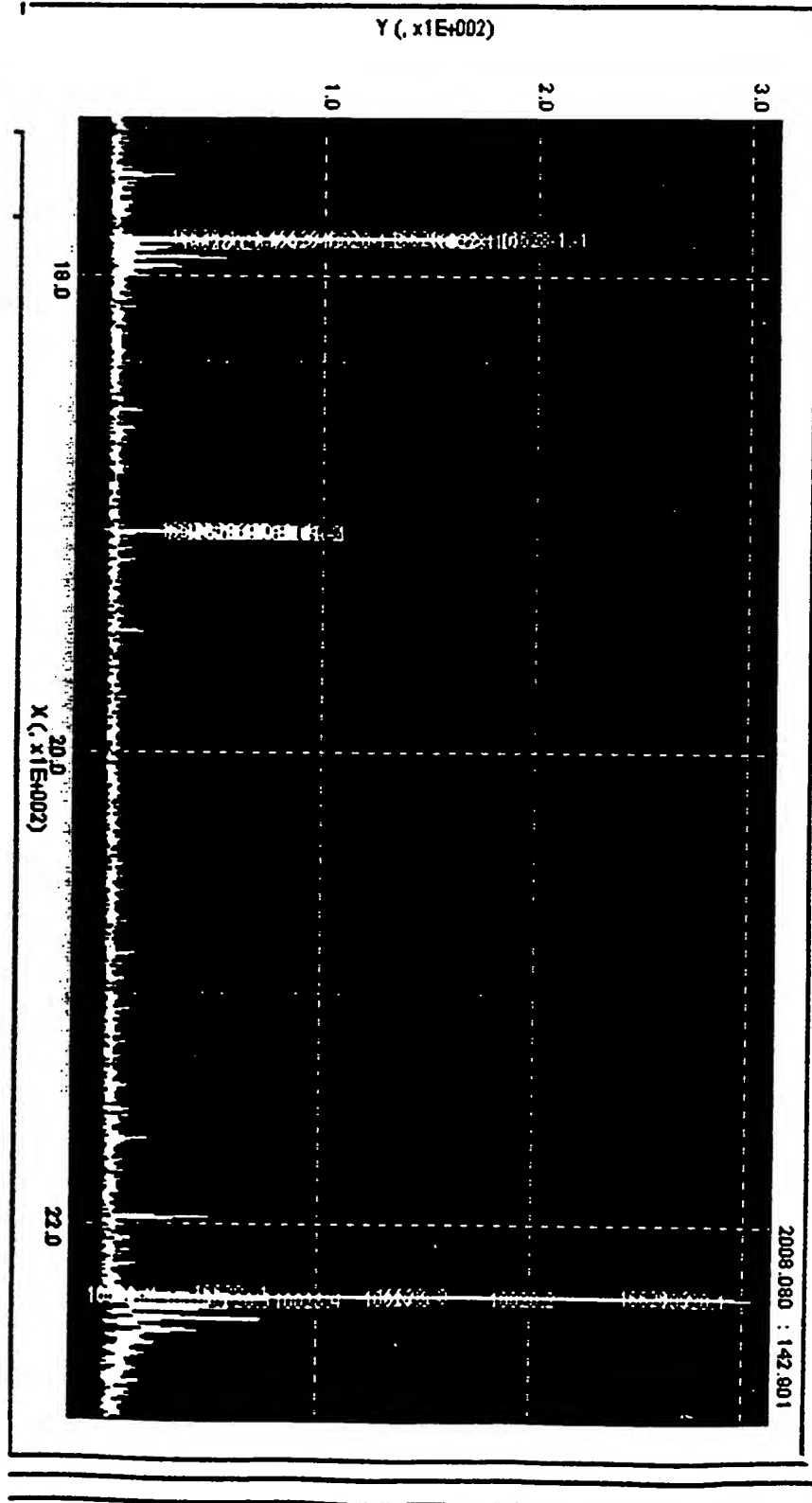


Figure 30

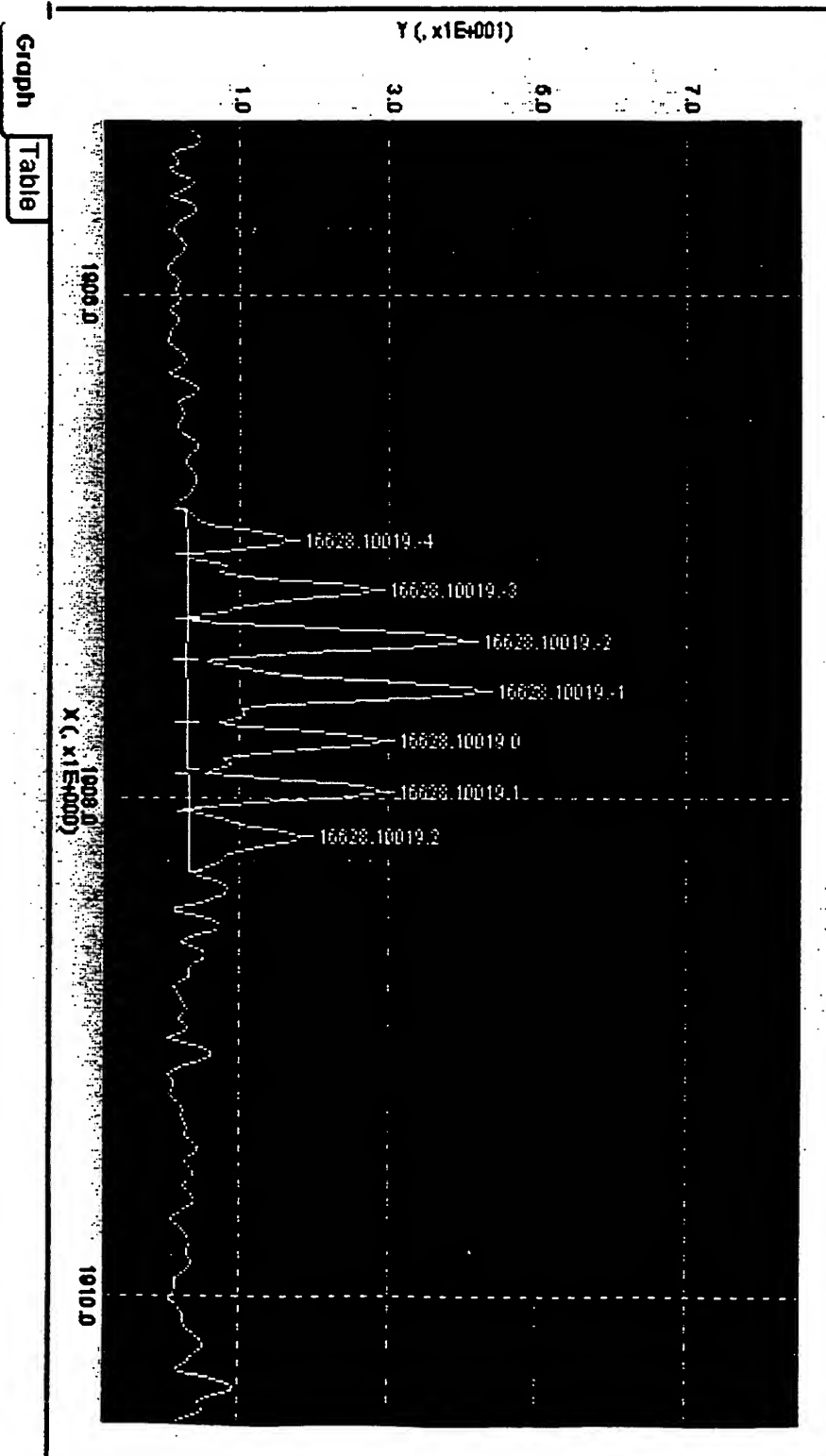


Figure 31

Figure 32

nr	name	apex	start	stop	height	area
1	16628-1.4	1783.710	1783.635	1783.834	14.55	1.63
2	16628-1.3	1783.909	1783.834	1783.972	60.04	5.15
3	16628-1.2	1784.109	1784.021	1784.184	115.60	11.14
4	16628-1.1	1784.308	1784.233	1784.383	167.34	15.89
5	16628-1.0	1784.508	1784.433	1784.620	133.94	14.74
6	16628-1.1	1784.707	1784.620	1784.795	136.60	13.38
7	16628-1.2	1784.907	1784.795	1784.982	82.63	8.56
8	16628-1.3	1785.107	1785.032	1785.219	57.81	5.21
9	16628-1.4	1785.306	1785.232	1785.369	32.31	2.65
10	16628-1.5	1785.506	1785.456	1785.569	17.67	1.12
11	16628.10019-.4	1906.974	1906.874	1907.031	12.63	1.00
12	16628.10019-.3	1907.173	1907.045	1907.273	22.54	2.11
13	16628.10019-.2	1907.373	1907.287	1907.444	33.86	2.91
14	16628.10019-.1	1907.572	1907.458	1907.701	34.87	3.30
15	16628.10019.0	1907.772	1907.701	1907.843	20.93	1.55
16	16628.10019.1	1907.972	1907.900	1908.043	21.03	1.55
17	16628.10019.2	1908.157	1908.086	1908.271	10.97	0.90
18	16628-.4	2229.874	2229.679	2230.029	27.51	4.87
19	16628-.3	2230.146	2230.029	2230.263	111.72	16.23
20	16628-.2	2230.380	2230.263	2230.516	225.18	32.39
21	16628-.1	2230.633	2230.516	2230.770	280.66	40.90
22	16628.0	2230.887	2230.770	2231.023	287.24	41.95
23	16628.1	2231.140	2231.023	2231.257	242.23	34.17

Graph Table

**Figure 33**

